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PATENT
Customer No. 22,852
Attorney Docket No. 5552.0265-04000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Heinz-Jürgen FRIESEN et al.	Group Art Unit: 1641
Application No.: 09/820,974	Examiner: Christopher L. CHIN
Filed: March 30, 2001	
For: SHEET-LIKE DIAGNOSTIC DEVICE	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

FOURTH SUPPLEMENTAL DECLARATION UNDER 37 C.F.R. § 1.175(b)

On behalf of Dade Behring Marburg GmbH, formerly Behringwerke Aktiengesellschaft, the assignee of the entire interest in U.S. Patent No. 4,861,711, (the '711 patent), we hereby declare that:

1. We have authority to sign documents on behalf of Dade Behring Marburg GmbH. Dade Behring Marburg GmbH is the owner of the entire right, title, and interest in and to United States Patent No. 4,861,711, granted August 29, 1989, by virtue of a name change from Behring Diagnostics GmbH to Dade Behring Marburg GmbH, which was recorded at Reel 9197, Frame 0667. Behring Diagnostics GmbH was the sole assignee of the patent by virtue of an assignment executed by Behringwerke Aktiengesellschaft, which was recorded at Reel 8842, Frame 0428. Behringwerke Aktiengesellschaft was the sole assignee of the patent by virtue of an assignment executed by inventors Heinz-Jürgen Friesen, Gerd Grenner, Hans-Erwin Pauly, Helmut

Kohl, Klaus Habenstein, and Joseph Stärk, which was recorded at Reel 4496, Frame 0646.

2. We believe that the original, first and joint inventors of the subject matter which is claimed in the above-identified reissue application and for which a reissue patent is sought on the above entitled invention are Heinz-Jürgen Friesen (citizen of Germany), Gerd Grenner (citizen of Germany), Hans-Erwin Pauly (citizen of Germany), Helmut Kohl (deceased), Klaus Habenstein (citizen of Germany), and Joseph Stärk (citizen of Germany). The above identified reissue application was filed on March 30, 2001, and was accorded Serial No. 09/820,974.

3. We have reviewed and understand the contents of the above-identified reissue specification, including the reissue claims.

4. We acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

5. We hereby claim foreign priority benefits under Title 35, United States Code, § 119, of the foreign applications for patent listed below, and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Country	Application No.	Date of Filing
Federal Republic of Germany	P 3445816	December 15, 1984

6. We executed an Original Reissue Declaration on May 7, 1996, for the parent Reissue Application No. 08/544,579, which was filed in this reissue application on March 30, 2001. In that declaration, we declared that "the '711 patent may be partly inoperative or invalid by claiming more than the patentee had a right to claim. Any error

that may have occurr d in claiming more than the patentee was entitled to ar se without any deceptive intent on the part of the inventors or Behringwerke." See Original Declaration ¶ 6. We then recited information regarding Interference No. 103,072 between Friesen et al. and Guire et al., in which the '711 patent was involved.

7. Claims 1 to 8, 10 to 21, and 23 to 34 of the '711 patent were held unpatentable to Friesen et al. in view of Guire et al. being awarded priority in Interference No. 103,072. Accordingly, we declare in this Fourth Supplemental Declaration that at least one error being relied upon as the basis for reissue under 37 C.F.R. 1.175(a)(1) is that the '711 patent is partly inoperative or invalid based on the findings of the Administrative Patent Judge in Interference No. 103,072, as claiming more than the patentee was entitled to claim in claim 1. We understand that when a claim is held unpatentable in view of a priority award to an opponent, the entire claim is held unpatentable. Thus, there is no particular portion of the claim language of claim 1 that constitutes an error. Rather, the entire claim language of claim 1 of the '711 patent constitutes the at least one error in the '711 patent.

8. In the present reissue application, the error in claim 1 has been corrected by adding claim 35. Claim 35 includes the subject matter of claim 1 of the '711 patent and further includes the language "wherein said MPAZ has dimensions to contain sufficient fluid to permit the fluid to migrate to the AZ, and wherein said layer of substantially planar zones contains at least two sheet-like strips made from different materials."

9. We believe that every error in the '711 patent being corrected in the present reissue application, including any error not covered by the original reissue declaration, the Supplemental Declaration, the Second Supplemental Declaration, or

the Third Supplemental Declaration submitted in this reissue application, arose without deceptive intent on the part of the inventors or the assignee, Dade Behring Marburg GmbH.

10. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the reissue application or any patent issued thereon.

Respectfully submitted,

Cynthia Tymeson (ppa)
Name: Cynthia Tymeson
Position: Corporate Counsel, Procurist

Dated: March 2, 2004

IN THE UNITED STATES PATENT OFFICE

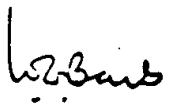
I, Wolfgang Gerson BARB, B.Sc., Ph.D., F.P.R.I., F.I.L., translator to Randall Woolcott Services plc of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That I am well acquainted with the German and English languages.

3. That the attached is a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in Germany on 15 December 1984 under the number P 34 45 816.6 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the patent application in the United States of America or any patent issuing thereon.



The 6th day of October 1992

FEDERAL REPUBLIC OF GERMANY

CERTIFICATE

BEHRINGWERKE AKTIENGESELLSCHAFT

of

3550 Marburg

have filed a Patent Application under the title:

"Sheet-like diagnostic agent"

on 15th December 1984 at the German Patent Office.

The attached document is a correct and accurate reproduction of the original submission for this Patent Application.

The attached abstract, which is to be appended to the Application but is not part of the Application, is identical to the original filed on 15th December 1984.

The German Patent Office has for the time being given the Application the symbol G 01 N 33/54 of the International Patent Classification.

Munich, 31st July 1985

President of the German Patent Office

pp

Maget

File No:
P 34 46 816.6

BEHRINGWERKE AKTIENGESELLSCHAFT

84/B 024 - Ma 518
Dr. Ha/Sa

Sheet-like diagnostic agent

The invention relates to a solid diagnostic agent which comprises several functional sectors and is used for the detection and quantitative determination of substances or analytes in biological fluids. The invention also relates to a process using this agent in which, after the agent has come into contact with the fluid, the analytes react with specific combination partners having biological affinity and are detected by means of labelling reagents.

In methods of diagnosis, the ability to identify and estimate specific compounds has made it possible to monitor the administration of medicaments, the quantification of physiologically active compounds or secondary products thereof and the diagnosis of infections. In this respect, the immunoassay methods (RIA, ELISA and the agglutination test) are of particular importance. The specific combination reactions utilized in the tests are not limited to immunological interactions, such as antigen-antibody or hapten-antibody interactions, but also utilize interactions having biological affinity, such as lectin-sugar or active compound-receptor.

Although the existing tests are sensitive and specific, they do not constitute convenient application forms, because of the long duration of the tests (in most cases several hours or even days) and the frequent test stages, such as immune reaction, washing stages and enzymatic reaction. The long test times are not compatible with use in emergency methods of diagnosis.

Integrated dry chemical test elements, such as are described in the present invention, simplify the performance of the tests and shorten the test times.

A sheet-like test element, in which all the components of the immune reaction of a heterogeneous immunoassay using solid phase detection, and the functional performance and the "bound-free" separation, are integrated is not (sic) described.

Whereas in the test strip assembly the immune reaction stages and the separation of bound and free phases are operated in the heterogeneous test by directed streams of liquid, in test element assemblies operating by means of thin layers laminated over one another (film technology), processes controlled by diffusion and directed by the concentration gradient are possible driving forces. A fluorescence labelling is used in German Offenlegungsschrift 3,329,728 (Japanese Patent P144,341/82) and EP A 0,097,952 (Japanese Patent 114,359/82). The labelling has a low molecular weight and hence promotes processes controlled by diffusion. However, the test has to be carried out at an elevated temperature. In the first of these two cases both the free phase and also the bound phase are evaluated. In film technology the absorption of solvent is effected either by hydrating swellable components or by filling capillary cavities. In the case of assemblies having layers laminated over one another only the top layer and the bottom layer are accessible to detection without major difficulties.

After the reaction stages have taken place it is difficult to react reagents with components in intermediately placed layers. In the test strip assembly having zones situated one behind another, such as is used in the present invention, in principle each zone is readily accessible, both from above and also from below, for a determination and also for the addition of reagents which may perhaps be required.

The invention relates to a sheet-like diagnostic agent which contains all the reagent components and which contains not only all the components required for the functional sequence, but also the functional sequences

themselves in an integrated form, and by means of which it is possible to detect an analyte having properties of biological affinity, a solution of the analyte being brought into contact with a functional region of the agent scheduled for this purpose, and the analyte being detected via a signal-producing system in a single functional region, a solid phase zone.

A second analyte, or further analytes, as constituents of the same solution can be detected at the same time by means of the agent, if these analytes possess properties of biological affinity different from the first analyte. They are also detected in the same manner as the first analyte in a single functional region, a solid phase zone appropriate for them. The functional regions for the detection of the second or further analytes are situated on the sheet-like agent in front of or behind the functional region for the detection of the first analyte. The agent can also contain several solid phase zones which are appropriate for an analyte and different measurement ranges of this analyte. The agent and its components are preferably in a dry form.

The sheet-like diagnostic agent comprises one or several strips, arranged behind one another, of material which have a capacity for absorbing aqueous solutions. The strips are fixed on a firm substrate. They contain the reagent components required for the particular diagnostic agent and thus become functional sectors or functional regions. The functional sector situated at one end of the strip-shaped agent (solvent application zone) is brought into contact with the analyte solution by being dipped into the latter or by the application of the latter. The solution migrates through all the functional regions. The absorptive capacity of the supporting materials of which the strips are composed causes a flow of liquid which ceases at the other end of the strip-shaped agent. The analyte can also be applied in the middle region of the agent, and a flow of liquid from one end of the agent to

the other can then be induced.

The sample does not have to be applied directly to the chromatographing section of the test strip. It can also be applied to an absorptive material which is situated on the test strip and has the function of removing blood cells from the sample. After being filtered the sample then reaches the test strip. In the course of this filtration process the addition of reagents can be effected at the same time by dissolving the latter out of components present in the filter in a dry state. Interfering factors can be eliminated from the solution by means of such components. Thus, for instance, the ascorbic acid present in a sample, which interferes in the use of oxidases and peroxidases as labelling agents, can be rendered harmless by means of a suitable oxidizing agent. Furthermore, the filter can also have the function of an adsorbent which removes interfering factors from the sample by adsorption. The filtration, adsorption and reagent admixing function for conditioning the sample for the test can also be taken over by the mobile phase application zone or a zone situated behind the latter.

The distribution of the solvent in the individual functional regions depends on the adsorptive capacity and the dimensions of the materials used.

The solvent application zone can have the function of a volume metering element, as described in German Patents 3,043,608 and 2,332,760, and US Patents 3,464,560, 3,600,306, 3,667,607, 3,902,847, 4,144,306 and 4,258,001. It can contain, in dry form, the various reagents required for the function of the test element. The solvent application zone can be a piece of fabric paper which is located at one end of the test element and which becomes completely saturated with a definite volume of liquid merely by being dipped into a solution, for example a solution of the sample, or by being briefly flushed with tap water, and then releases the liquid to the succeeding

zones more slowly and in a controlled manner. The solvent application zone has dimensions such that it gives up sufficient liquid to permit the latter to migrate to the other end of the agent, the end of the absorption zone.

Between the solvent application zone and the absorption zone there are located the functional regions in which are contained reaction components for the performance of the test and in which all the reaction stages of the performance of the test take place. Part of the reaction components for the performance of the test can also be housed in the sample application zone. The absorption zone has the function of absorbing excess and freely mobile reagent components and reaction products of the signal-producing system.

The absorbent supporting materials in the form of one or more strips, as constituents of the various functional regions, can, according to choice, be composed of cellulose, of chemical derivatives of cellulose or of plastics having a porous or fibrous structure and adequately hydrophilic properties, or of particles such as cellulose or silica gel embedded in a plastic membrane, and also of natural products which are hydrophilic but have been rendered insoluble in water. A combination of strips composed of different materials can be used. Suitable absorbent materials are selected on the basis of the requirements set for the particular diagnostic agent.

In various embodiments of immunochemical, diagnostic agents, the reagent components having biological affinity are antigens, haptens or antibodies. In the event that glycoproteins or oligosaccharides which attach themselves to lectins are to be detected, one reactant having biological affinity can be the specific lectin, while the second reactant having biological affinity can be an antibody which is directed against a point of attachment on the analyte other than that of the lectin. In the event that microbial active compounds are to be detected,

one combination partner can be the receptor substance for the active compound, while the second combination partner can be an antibody which is directed against another point of attachment on the active compound.

One combination partner having biological affinity becomes attached during the progress of the reaction, or has already been attached, to the supporting material in the functional region scheduled for the detection of the analyte (solid phase zone). It is also called the solid phase combination partner. The other combination partners are present in the supporting materials. They are provided with a labelling.

Amongst the various known possibilities of labelling, enzyme labelling is preferred. It requires chromogenic substrate systems or substrate systems which produce fluorescence or chemiluminescence. Chemiluminescence labelling represents a further example of a labelling which is only measured after the addition of a reagent. It is possible to measure either the chemiluminescence itself or a fluorescence excited by the latter. In most cases fluorescence labelling is measured without the addition of a reagent being required. However, as in the use of certain rare earth chelates, it can also be desirable to produce the fluorophore to be measured only as the result of adding a reagent, or to add a second fluorophore which becomes excited by the first or which excites the first fluorophore. The fluorescence can be measured at one point, as a function of time or as fluorescence polarization.

A reagent required for detection can be induced to react with the immune complex to be detected in various ways, after the separation stage. Part of the signal-producing system can be located in the solid phase zone. After the solid phase has been adequately washed, a reagent required to detect the labelling can be released at a retarded rate in various embodiments in the heterogeneous

immunoassay with detection in the bound phase. The following are possible examples:

The application of reagents by means of a stream of liquid arranged parallel to the main stream of liquid, but flowing more slowly and starting from the mobile phase reservoir and entering in front of the zone containing the labelled component. The parallel stream of liquid can be controlled by using an absorbent medium which chromatographs more slowly, for example a paper which chromatographs suitably slowly or a paper which is impregnated in places with "components temporarily blocking the way", such as, for example, polymers which impart a high viscosity on passing into solution (for example polyvinyl alcohols or dextrans).

After the solid phase has been adequately washed (= completion of chromatography), the application of reagents can be effected by pressing down an element which is a solid constituent of the test element. The "pressing down" can be effected mechanically or by removing distance pieces by the action of a stream of liquid. For example, the mechanical pressing down of an element containing the reagents can be effected by pressing down a flap or a piece of paper supported by distance pieces. The lowering of an element containing the reagents by the action of the stream of liquid can be effected, for example, by laminating over one another the solid phase, a water-soluble polymer and the reagent carrier (for example a suitably impregnated piece of paper).

A retarded introduction of reagents into the liquid stream can be effected using a microencapsulated reagent which only emerges from the encapsulation after the solid phase has been adequately washed, or by coating the reagent adhering in the matrix with components which dissolve slowly.

One possible means presented for the special case of enzyme labelling is as follows: when a peroxidase labelling is used, a glucose oxidase zone can be placed in front of the solid phase zone. Glucose and also the chromogen are then incorporated into the liquid stream, which can result in color formation behind the glucose oxidase. Appreciable color formation is only observed if, at an appropriately high concentration of peroxidase, sufficient H_2O_2 is formed by the oxidase. This formation of the peroxide sets in slowly, reaches an optimum concentration and finally reaches a high concentration which results in inhibition of the enzyme and thus automatic cessation of the color formation. This coloration can be moderated if an H_2O_2-acceptor, for example a thioether as a mild reducing agent, or the enzyme catalase is incorporated in the oxidase zone or in front of the latter.

In this example a reagent for detecting the labelling is produced by a delay circuit, making use of an enzyme. The color formation in the solid phase zone only begins after this zone has been adequately washed free from non-specifically bound labelling by the stream of liquid.

There are several possible means of preparing the solid phase zone. The components fixed there can be attached by chemical covalent bonds or adsorptively to an absorptive support which is a part of the test element. These components can also be attached to a dispersion of particles which remains fixed at the place of application after it has been applied to an adsorbent support. For example, suspensions of cells carrying specific receptors on their surface, such as, for instance, Staphylococcus aureus Cowan I cells, or latex particles carrying combination partners of biological affinity attached to their surface, are suitable for being fixed in a paper matrix. The components of the test strip which are attached to pipettable supports and also the unattached

components of the test strip can be dried onto the absorbent matrix of the element by air drying; freeze-drying stages are not absolutely necessary.

A few test performances will be illustrated as examples of embodiments which can be regarded as independent of the labelling used. For the sake of simplicity, they are only described for the detection of a single analyte by means of the diagnostic agent.

The following two embodiments, which conform to the principle of competitive immunoassay, will be described for the case where the analyte has only a single combination point of biological affinity or only one combination point of biological affinity out of several is utilized:

The solid phase combination partner is attached by covalent bonds or adsorptively to the supporting material of the solid phase functional region. The solution of analyte renders mobile a predetermined amount of labelled analyte contained in the diagnostic agent. The two components migrate into the functional sector containing the solid phase combination partner and compete for combination with the solid phase combination partner. If the proportion of analyte is high compared with the labelled analyte, little labelled analyte will be attached. If it is low, a great deal of labelled analyte will be attached.

The solid phase combination partner is housed as an unattached component in a functional region in front of the solid phase functional region. The oncoming front of solvent transports it into the solid phase functional region, where it becomes attached. This solid phase attachment is produced by combination systems of biological affinity which are independent of the combination system of the analyte. A combination partner which is conjugated with biotin attaches itself to avidin attached

to the support. An immunoglobulin, such as IgG, as a combination partner, is fixed via its Fc component to support-attached protein A of S. aureus, or is attached by a support-attached, non-genotypical antibody.

As previously described, the analyte and the labelled analyte compete, as constituents of the diagnostic agent, for the attachments to the solid phase combination partner during the progress of the function. This competition reaction takes place partly with the dissolved solid phase combination partner and partly with the solid phase combination partner which has already been attached to the solid phase.

If two combination points of differing specificity are present in an analyte, several embodiments, conforming to the principle of sandwich immunoassay, of the diagnostic agent are conceivable. Two of these will also be illustrated below:

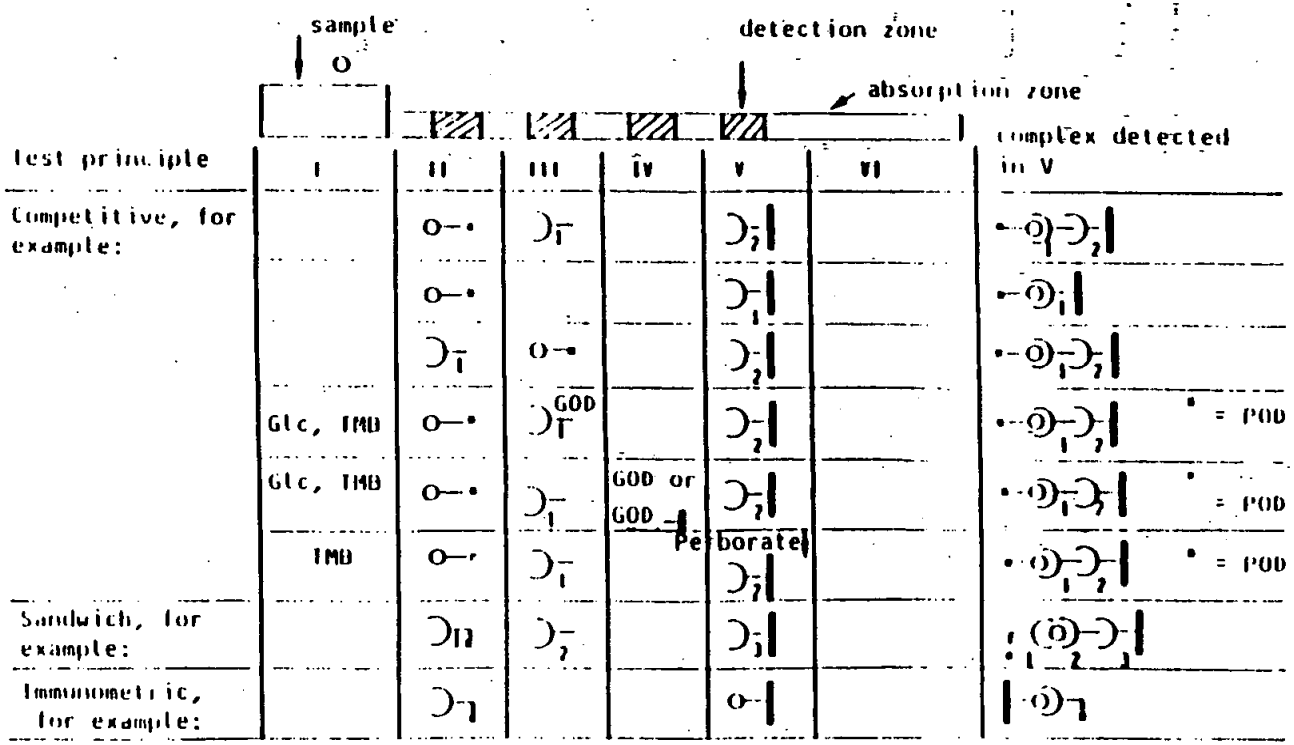
If the solid phase combination partner is attached by covalent bonds or adsorptively to the supporting material of the solid phase functional region, the analyte forms, with the labelled combination partner, a binary complex which migrates together with the solvent into the solid phase functional region and reacts there with the solid phase combination partner, with the formation of a ternary complex, attached to the solid phase, which can be detected via the labelling of the first combination partner. The excess labelled combination partner is removed by the solvent into the subsequent functional region, the absorption zone.

If the solid phase combination partner is present in a non-attached form in the diagnostic agent and is rendered mobile by the solvent, the two reactants of the analyte of biological affinity are housed in the functional regions in such a way that the analyte reacts simultaneously or successively with both reactants and the resulting

ternary complex then migrates into the solid phase functional region, where, as already described above, it becomes attached to the solid phase via a second system of biological affinity which is independent of that of the analyte.

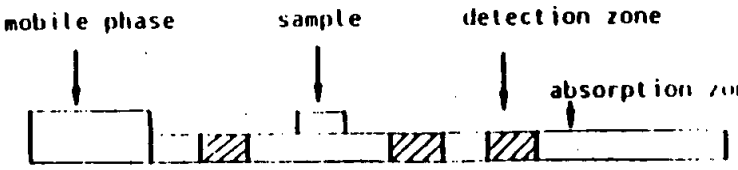
In order to illustrate the embodiments described above and further embodiments which conform to the immunometric test principle, the principle of indirect antibody detection or the ELA (enzyme-labelled-antigen) principle of immunoassay, summary tables I and II illustrate in an exemplary manner the distribution of the components of the agent in the functional regions and, after the performance of the reaction, the composition of the solid phase complex, the amount of which is a measure of the concentration of analytes in the sample.

SUMMARY TABLE I: EXAMPLES OF TEST ASSEMBLIES WITH SAMPLE OR WITH PREVIOUS DILUTION OF SAMPLE IN THE FORM OF MOBILE PHASE



for explanation of symbols see summary table II

SUMMARY TABLE II: EXAMPLES OF TEST ASSEMBLIES HAVING A SEPARATE MOBILE PHASE



Test principle	I	II	III	IV	V	VI
Competitive, for example:						
Sandwich, for example						
Immunometric, for example						
Indirect detection of antibodies						
EIA (enzyme-labelled antigen)						

GOD glucose oxidase; POD = peroxidase; IMB = tetramethylbenzidine;

Glc α-D-glucose

Delivery of the component X to the particular zone

component attached to solid phase

attaching component (receptor)

antibody or receptor having combination points for another receptor

labelling; () = component which can be attached by a receptor

It has been found that a completely integrated test strip operating in accordance with the principle of heterogeneous immunoassay by means of solid phase detection is not only feasible in principle, but can, in addition, also be evaluated within a period of less than one hour, the quantification and the sensitivity of conventional RIAs or ELISAs being achieved. The detection of trace components in the range of 10^{-12} mol/liter has been made possible at reaction times of less than 30 minutes, at room temperature, the amounts of sample required being 10^{-16} mol, corresponding, for example, to approx. 1 pg. The arrangements described also enable tests of lower sensitivity requirements to be carried out, however. Standard curves over two to three decades were obtained when evaluation was carried out with the Sanoquell reflectometer (made by Quelle). The chromatography time for the test element, including complete color development, is not more than 16 minutes. Evaluation can also be carried out visually. With HCG as analyte, the start of the range of determination in an example using a glucose oxidase attached to a solid phase and a peroxidase labelling was 0.3 mg/ml (corresponding to 3 U/liter).

In the example following, the application of the principle of the competitive double antibody test is presented as a concrete embodiment. In this test configuration, four components have to be reacted successively for the determination reaction and the separation stage, the reaction times and the concentrations of the reactant being critical values. The example is not to be regarded as limiting in any way, but merely serves to illustrate the subject of the invention further.

Example

Completely integrated enzyme-immunochemical test strip for the detection of HCG by means of a built-in chromogen substrate system.

1.1. Reagents

1.1.1. HCG-perodixase conjugate

HCG having a specific activity of approx. 3000 U/mg was obtained from Organon. Peroxidase from horseradish was obtained from Boehringer Mannheim (catalog no. 413,470). The hetero-bifunctional reagent N-δ-maleimidobutyryloxy-succinimide (GMBS) was obtained from Behring Diagnostics and was reacted with the HCG as described by Tanimore et al., 1983, in J.Imm. Meth. 62, 123-131. 2-iminothiolane hydrochloride (Sigma, catalog no. I 6256) was reacted with peroxidase as described by King et al., 1978, in Biochemistry 17, 1499-1506. A conjugate was prepared from the GMBS-HCG and the iminothiolane-peroxidase as described by Tanimori et al. The crude conjugate was purified by gel chromatography over Ultrogel ACA 44 (LKB). The fraction in which about 1-2 peroxidase molecules were coupled per HCG molecule was used for the test. The conjugate was diluted with Enzygnost IgE incubation medium made by Behringwerke, order no. OS D, designated briefly as incubation medium in the following text.

1.1.2. Antibodies

Antibodies against HCG were obtained by immunizing rabbits, and antibodies against rabbit-IgG were obtained by immunizing goats. The IgG fractions were isolated from serum by ammonium sulfate precipitation and anion exchange chromatography, and were purified further by immunoadsorption. The methods used are described in the book "Immunologische Arbeitsmethoden" (Immunological working methods), Helmut Friemel, Editor, 1984, Gustav Fischer Verlag, Stuttgart. The anti-HCG antibody was finally diluted in the conjugate dilution buffer indicated above.

1.1.3. Glucose oxidase

Glucose oxidase from Aspergillus niger was obtained as a

solution containing 300 U/mg (Serva, catalog no. 22,737). The glucose oxidase was finally diluted with incubation medium.

1.1.4. Glusoce and tetramethylbenzidine

α-D-glucose and tetramethylbenzidine hydrochloride were obtained from Serva, catalog no. 22,720 and 35,926, respectively.

1.2. Preparation of the agent

The sheet-like functional regions were prepared as follows:

The mobile phase application zone was prepared by cutting, to dimensions of 20 x 6 mm, a fabric sponge cloth made by Kalle; this is a synthetic sponge of regenerated cellulose which has been compressed in a dry state. It was impregnated with a solution of 50 mg of glucose and 0.75 mg of tetramethylbenzidine hydrochloride per ml of water, and was dried in a stream of air.

The conjugate, the anti-HCG antibody and glucose oxidase (5 µl of each at 25 µl/ml, 100 µl/ml and 0.1 mg/ml, respectively) were applied behind one another, at uniform distance, to a 45 x 5 mm piece of MN no. 1 paper (Macherey & Nagel), and were dried in the air.

A piece measuring 5 x 5 mm of Schleicher & Schüll no. 597 paper was coated in a covalent manner with anti-rabbit IgG-antibody as the solid phase zone. This was effected by coupling the antibody with the paper, which had been activated with cyanogen bromide, as described by Clarke et al., 1979, Meth.Enzymology, volume 68, 441-442.

A 20 x 5 mm piece of Schleicher & Schüll no. 2668/8 paper was used as the absorption zone.

The four pieces of paper, with a 0.5 - 1 mm overlap behind one another, were fixed on a firm substrate by means of double-sided adhesive tape (Tesaband made by Beiersdorf), so that a test strip 5 mm wide was formed.

1.3. Performance of the test

The test was carried out in each case by applying 200 µl of an HCG dilution in incubation medium to the fabric.

1.4. Results

The chromatographic development of the test element and the self-actuating color development were complete after 15 minutes at room temperature, and evaluation could be carried out either visually or by means of a reflectometer.

The following values were obtained when evaluating the solid phase zone (no. 597 paper) with the Sanoquell blood glucose evaluation apparatus made by Quelle:

HCG concentration (U/liter)	Measured values (mg of glucose per dl of blood)
0.3	107
3	117
30	95
300	70
3000	0

The following values were obtained with the same test strips using the Rapimat urine test strip evaluation apparatus made by Behringwerke:

HCG concentration (U/liter)	Measured values (BIT)
0.3	76
3	76
30	94
300	119
3000	135

The test strip assembly shown here can also be achieved if the glucose oxidase and the anti-HCG antibody are located in the same zone. The test strip, which is correspondingly shorter, then has a test time of approx. 10 minutes.

Patent Claims

1. An analytical agent for the detection or determination of a component of a combination pair having biological affinity (analyte) in a fluid, composed of several sheet-like zones which are arranged behind one another and are in adsorbent contact with one another through their edges, containing a mobile phase application zone (MPAZ) at one end of the agent and an adsorption zone (AZ) at the other end and also further adsorptive zones situated intermediately in which reactants capable of interactions, of biological affinity, with the analyte are arranged in such a way that reactants capable of reacting with one another are present, separated spacially, wherein

a) a reactant is fixed to the solid phase zone (SPZ) by means of covalent bonds or adsorptively or via an interaction of biological affinity in a zone which is located between the MPAZ and AZ and is in contact with the AZ, or becomes attached in a reaction which takes place in the agent through a further reactant which is fixed in the SPZ by covalent bonds or adsorptively or via an interaction of biological affinity,

b) a further labelled reactant (conjugate) is located, unattached, in a zone between the MPAZ and the SPZ, and

c) the analyte application zone is the MPAZ or a zone between MPAZ and AZ.

2. A sheet-like diagnostic agent as claimed in claim 1 for the detection of two or more analytes each of which has one or more attachment points of biological affinity, which contains, per analyte, a spacially separated solid phase zone which is provided with combination partners attached to the support and specific for the particular analyte, and in which agent the analytes are detected separately.

3. An agent as claimed in claim 1 or 2, wherein the MPAZ has the function of a volume metering element and releases to the subsequent zones at least sufficient liquid for the liquid, controlled by capillary forces, to reach the end of the AZ.

4. An agent as claimed in one of claims 1 to 3, wherein the MPAZ is a plastic sponge or a particulate layer which is composed of hydrophilic polymers and which can, if appropriate, contain chemicals, buffer substances or other substances required for the test.

5. An agent as claimed in one of claims 1 to 4, wherein the sample application zone retains blood cells.

6. An agent as claimed in one of claims 1 to 5, wherein the sample application zone is laminated onto one of the sheet-like zones of the chromatographing section of the agent and is in adsorptive contact with this zone.

7. An agent as claimed in one of claims 1 to 6, wherein all or some of the reagents required for the detection of the labelling are present in one or more of the sheet-like zones of the agent or in a zone which is laminated onto one of the sheet-like zones of the chromatographing section of the agent and is in adsorptive contact with this zone.

8. A process using an agent as claimed in one of claims 1 to 7, wherein the reactants present in the agent are in a dehydrated form and are rehydrated or solvated by the liquids fed to the agent.

9. The process as claimed in claim 8, using an agent according to one of claims 1 to 7, wherein, after the liquid sample containing the analyte has been fed to the MPAZ or after the sample has been fed to a sample application zone and a mobile phase has been fed to the MPAZ, the liquid reaches the end of the AZ, under the control of capillary forces, and reactions between reactants contained in the agent and the analyte are thereby set in operation, and, after the labelling which is not specifically attached to the solid phase has been removed chromatographically, the amount of the labelling in the solid phase zone, which is a measure of the analyte concentration in the sample, is determined.

10. The process as claimed in claim 8 or 9, using an agent as claimed in one of claims 1 to 7, wherein the reactions taking place in the agent are based on the

principles of immunological detection reactions, of competitive immunometric or sandwich immunoassay or of indirect antibody detection by means of a labelled antibody or of antibody detection by means of a labelled antigen.

11. The process as claimed in one of claims 8, 9 or 10, using an agent as claimed in one of claims 1 to 7, wherein the labelling agent is a fluorophor which is detected or measured directly or is detected or measured after the addition of a reagent present in the agent, or a fluorophor which is detected or measured directly or after the addition of a further reagent is formed from the labelling agent by the addition of a reagent present in the agent.

12. The process as claimed in one of claims 8 to 10, using an agent as claimed in one of claims 1 to 7, wherein the labelling agent is a compound which can be excited to give chemiluminescence, it being possible to detect or measure chemiluminescence after the addition of a reagent present in the agent.

13. The process as claimed in claims 8, 9 or 10, using an agent as claimed in one of claims 1 to 7, wherein the labelling agent is an enzyme the activity of which is determined with the aid of a reagent present in the agent.

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Abstract of the disclosure

Sheet-like diagnostic agent

A solid diagnostic agent for the quantitative determination of substances of biological affinity in biological fluids is described. A process is also described in which the biological fluid is brought into contact with a specific functional sector of the agent, the fluid migrates through several functional sectors situated beside one another and containing suitable reagent components, and one or more substances of biological affinity are detected in such functional sectors which contains (sic), for each substance to be detected, at least one combination partner of biological affinity, attached to a solid phase.